Perspectives in Science (2015) 6, 27—37

ScienceDirect

journal homepage: www.elsevier.com/pisc

A brief history of circadian time: The emergence of redox oscillations as a novel component of biological rhythms☆

Lisa Wulund, Akhilesh B. Reddy*

Department of Clinical Neurosciences, Wellcome Trust-MRC Institute of Metabolic Science, University of Cambridge, Addenbrooke's Hospital, Cambridge CB2 0QQ, UK

Received 28 July 2015; accepted 20 August 2015
Available online 23 October 2015

KEYWORDS
Circadian rhythms;
Biological clocks;
Redox oscillations;
Chronobiology;
Metabolism

Summary Circadian rhythms are present in all living organisms. They organise processes such as gene transcription, mitosis, feeding, and rest at different times of day and night. These rhythms are orchestrated by a network of core 'clock genes' that are organised into transcription—translation feedback loops (TTFLs), producing oscillations with a period of approximately 24 h. The modern understanding of circadian timekeeping has revolved around the TTFL paradigm. Recently, however, this has been challenged by new findings that redox reactions persist in the absence of gene transcription, and that cycles of oxidation and reduction are conserved across all domain of life. These results suggest that non-transcriptional processes such as metabolic state may interact and work in parallel with the canonical genetic mechanisms of keeping circadian time.

Contents

☆ This article is part of a special issue entitled ''Proceedings of the Beilstein Bozen Symposium 2014 — Chemistry and Time''. Copyright by Beilstein-Institut www.beilstein-institut.de.

* Corresponding author. Tel.: +44 1223 338780.
E-mail address: areddy@cantab.net (A.B. Reddy).

http://dx.doi.org/10.1016/j.pisc.2015.08.002

Introduction

Biological timing is a necessity in all organisms, driven by the need to adapt to changes in the environment. Living species from bacteria to plants to humans exhibit cycles in physiology and behaviour over periods as short as seconds (*e.g.*, in the case of cardiac pace-making cells) and up to as long as months (*e.g.*, in the case of seasonal oscillators). The study of circadian rhythms examines 24-h oscillations in biological processes at the molecular, cellular, and behavioural levels. These processes have been shown to orchestrate basic cellular functions such as gene expression and protein translation, and can even be experienced as the daily rituals that make up the temporal fabric of human life — feeding activity and the onset of sleep. It is widely believed that these mechanisms evolved as a response to the rotation of the planet — cycles of day and night result in changes of ambient light level and temperature in the environment, and therefore evolutionary benefit is conferred to those organisms that can correctly anticipate and synchronise their physiology.

To this day, circadian clocks remain one of the most robust experimental systems wherein perturbations of genetic background or environmental state can be directly linked to changes in physiology and behaviour. Over 40% of all protein-coding genes have 24-h rhythms of gene *transcription* (Zhang et al., 2014) — the process of copying a gene into messenger ribonucleic acid (mRNA) that is then *translated* into proteins, which are the final arbiters of genetic coding. These gene expression rhythms in turn drive the oscillation of a wide swath of other biological processes including blood pressure, body temperature, blood hormone levels, and energy homeostasis (Hastings et al., 2003; Huang et al., 2011). Chronic disruption of normal circadian rhythms by shift-work and jet lag leads to physiological dysfunction which can manifest as mood disorders, cancer, cardiovascular disease, and metabolic diseases such as diabetes and obesity (Scott et al., 1997; Healy et al., 1993; Scheer et al., 2009; Hansen, 2001; Hansen and Stevens, 2012; Spiegel et al., 2009). Even small disruptions such as daylight savings can be associated with incidences of myocardial infarction (Janszky and Ljung, 2008; Janszky et al., 2012).

Circadian rhythms are ubiquitous in both multicellular and unicellular organisms. However, research in this area for the past few decades has largely focused on the oscillatory expression of genes and proteins. In this article, we will examine the predominant mechanistic model of circadian oscillation and explore recent discoveries that link the cell's transcriptional activity to its metabolic state. By doing this, we hope to provide insight for how the physical realities of night and day have shaped the evolution of life on this ever-rotating planet.

Chronobiology: constructing a biological clock

Circadian rhythms have been known to science for centuries, with the first recorded observation of biological timekeeping in 1729 stemming from the French astronomer Jean-Jacques d'Ortous de Mairan, who noted that the leaves of the *Mimosa* plant moved with a periodicity of 24-h, even when the plant was moved to a basement without light (Roenneberg and Merrow, 2005). The persistence of rhythms in a state of constant darkness means that the biological clock underlying the movements was 'endogenous' — even without an external stimulus the oscillation persists, *i.e.*, the clock was still ticking. In order for a clock to function, it must also be able to reset itself, and therefore, circadian rhythms in an organism must adapt to changing solar cycles throughout the seasons. Thus, the clock must be 'entrainable' by environmental cues which tell it set itself to the correct time. In mammalian systems the most robust synchronising cues or 'zeitgeber' — 'time-givers' in German — are light and food (Roenneberg and Merrow, 2005; Pittendrigh, 1960).

The mechanisms responsible for driving these observed circadian rhythms remained obscure until the advent of mutation screening in *Drosophila melanogaster*, in which it was discovered that alterations of the *period* (*Per*) gene altered the periodicity of locomotor activity (Konopka and Benzer, 1971; Reddy et al., 1984). Further studies elucidated more genes that altered the timing of behaviour, and homologues in mammals were subsequently discovered. Thus emerged the concept of 'core clock genes' — that the basis of biological timekeeping, the gears of the clock, consisted of just a handful of important genes. The mechanism driving these rhythms was then proposed to be a negative feedback loop (Hardin et al., 1990), and a model, known as the transcription—translation feedback loop (TTFL), has since become the dominant paradigm for conceptualising circadian oscillations in both plants (Gardner et al., 2006) and animals (Reppert and Weaver, 2002).

Negative feedback loops provide a basic explanation of how component elements, through negative autoregulation, generate oscillations; circadian biology is certainly no different to other purely physical or chemical systems. The proteins translated from the genes *Bmal1* (also known as *Arntl1*) and the aptly named *Clock* function as activators of transcriptional drive, resulting in the transcription of the *Period*, *Cryptochrome* (*Cry*), and *Rev-erb* families of genes. These genes then proceed to inhibit their own function — the REV-ERB proteins inhibit *Bmal1* transcription and the PER/CRY heterodimers repress the activity of the BMAL1/CLOCK heterodimer. Transcriptional decline of these genes and the actions of the retinoic acid-related orphan receptor (ROR) family of proteins eventually results in the removal of the inhibition of *Bmal1* transcription, and the feedback loop begins anew (Reppert and Weaver, 2002; Sato et al., 2004).

One fascinating feature of the TTFL is that its components are not conserved across the eukaryotes (organisms that contain a nucleus) — although the characteristic negative autoregulation remains, the genetic components often share little homology. For instance, the clock genes in the fungus *Neurospora crassa*, *white collar* (wc) and *frequency* (*frq*), do not resemble their mammalian counterparts *Bmal1* and *Clock*. Similarly, in the plant *Arabidopsis thaliana*, the only shared components of circadian timekeeping are the involvement of the *Cryptochromes* and the *Casein kinases* (Gardner et al., 2006). These genetic differences suggest that circadian timekeeping may have evolved differently in the separate phylogenetic kingdoms.

By necessity, the TTFL requires a nucleus, where transcription takes place. Therefore, it was assumed that prokaryotic organisms lacked a circadian rhythm. Since their cell cycle is much shorter than a 24-h period, it was long accepted that they simply did not need to synchronise their biological time to the day/night cycle. Cyanobacteria, however, obtain their energy through photosynthesis and thus, have a critical need that is tied to the timing of the sun. Indeed, in the 1980s it was discovered that a circadian clock was responsible for timing differential gene expression in two metabolic functions of the *Synechococcus* cyanobacterium: genes involved in photosynthesis were activated during the day and those involved in nitrogen fixation were active at night (Huang et al., 1990; Mitsui et al., 1986). A cluster of three adjacent genes, *kaiA*, *kaiB*, and *kaiC* were discovered to be essential for the generation of these rhythms. A simplified TTFL model was proposed to be the mechanism underpinning these oscillations with KaiA as the positive regulator of *kaiB* and *kaiC*, and KaiC serving as the negative autoregulator of the loop (Ishiura et al., 1998).

The TTFL model of circadian oscillations continued to dominate the field of circadian biology until 2005, when Kondo and colleagues revealed that independent of transcription and translation processes, rhythms of phosphorylation of KaiC were able to persist. This type of modification occurs after proteins have already been produced *via* translation, and are thus termed *post-translational modifications*. Astonishingly, *in vitro* reconstitution of the bare components necessary for the clock — the three Kai proteins and the phosphate donor adenosine triphosphate (ATP) — was able to produce a self-sustained 24-h oscillation of auto- and de-phosphorylation. They concluded that the phosphorylation state of KaiC served as the primary pacemaker for the cyanobacterial clock (Nakajima et al., 2005), and demonstrated for the first time that gene transcription was not a necessary condition for biological timing. However, the Kai-based phosphorylation model of non-transcriptional oscillators is limited, since the *Kai* genes in cyanobacteria do not share homology with the core clock genes identified in the TTFL of other species, and are not even found in other eubacteria (Kondo and Ishiura, 2000). Therefore, this model cannot represent a shared evolution of the bacterial clock mechanism, though it did open the door for scientific study into other post-translational processes that might interact with the circadian clock in higher species.

Casein kinases and protein phosphatases (which add or remove phosphate groups from proteins, respectively) are conserved across some phyla, and have been found to be regulate the cellular localisation and degradation of both the positive and negative components of the TTFL in *Arabidopsis*, *Neurospora*, *Drosophila*, mammals, and even humans (Sugano et al., 1998; Akten et al., 2003; Yang et al., 2002, 2004; Keesler et al., 2000; Sathyanarayanan et al., 2004; Lowrey et al., 2000). Mutations in the activity of casein kinase 1ε and 1δ lead to shortened periodicity of rhythms (Mehra et al., 2009), which is attributed to the increased turnover of PER2 (Meng et al., 2008). The majority of the activity of the casein kinases is not rhythmic, though there is a circadian pattern of expression of the regulatory subunits of *Drosophila* protein phosphatase 2A (Sathyanarayanan et al., 2004). In addition to being modified with addition/removal of phosphate groups, core clock components such as BMAL1 are known to be modified by the small ubiquitin-like modifier (SUMO) (Cardone et al., 2005), which leads to increased ubiquitinylation and enhanced transcriptional activity (Lee et al., 2008). It is generally believed that these post-translational mechanisms exist in order to fine-tune the timing of the clock — mediating phase shifts or altering the periodicity generated by the network — but is not a mechanism that generates the circadian rhythm of the cell, or organism as a whole.

In addition to transcriptional drive, the positive arms of the TTFL are now known to have DNA remodelling activity. DNA is normally packaged into dense chromosomes by special proteins called histones, which can be modified chemically to compact and decompress DNA so that it is inactive or active respectively. CLOCK, for example, is known to function as a histone acetyltransferase (Doi et al., 2006), and this acetylation is required for the rhythmic expression of other core clock and output genes, including its partner BMAL, which undergoes rhythmic acetylation in the liver (Hirayama et al., 2007a). The Sirtuin (SIRT) family of proteins can function as nicotinamide adenine dinucleotide (NAD)-dependent deacetylases, directly linking the energy state of the cell to histone lysine residue deacetylation. One family member, SIRT1, has been shown to interact with CLOCK and BMAL (Nakahata et al., 2008), and is also involved in the rhythmic deacetylation of the PER2 protein (Asher et al., 2008). NAD is a central metabolite, and is required for the oxidation of glucose in respiration reactions such as glycolysis, a fundamental energy production pathway. These results provide the first insights into how metabolic state and the circadian clock may be linked.

The interplay between metabolism and the circadian pacemaker

Mechanistic links between the redox state and the TTFL framework of circadian rhythms have begun to appear in a variety of organisms — from bacteria to flies to mammals. Although a single model has yet to emerge, studies strongly suggest that redox state may be an oscillation that feeds back upon the TTFL, whereby a cell's redox state may alter clock gene expression and the clock genes, in turn, regulate redox state.

Reduction and oxidation are the foundation of chemical reactions. The redox state of a cell is a careful balance

between the generation of oxidants through metabolic processes, and the amount of reducing agents available. Oxidants such as reactive oxygen species (ROS) are damaging to cellular components. In order to compensate, all organisms have evolved mechanisms to buffer oxidants, such as superoxide dismutase (SOD) and catalase, which decomposes H_2O_2 into water and oxygen. Glutathione (GSH) maintains the reduced state of protein sulphydryl groups that are necessary for DNA repair and prevents oxidative damage to cell membranes by reducing lipid peroxides (Valko et al., 2007). When oxidised, GSH forms a dimer (GSSG) that is reduced by the glutathione reductases (GR) *via* an NADPH-dependent reaction (Valko et al., 2007). Otherwise, GSSG is potentially toxic to the cell (Filomeni et al., 2003; Park et al., 2009). Glutathione peroxidases (GPx) are also antioxidant enzymes known to have an affinity for lipid peroxides, and may be involved in intracellular signalling. In other both fruit flies (Beaver et al., 2012) and mammals (Baydas et al., 2002; Hardeland et al., 2003), daily rhythms in the expression of GSH, and in the activity of SOD, GPx, and GR have been observed.

Since cyanobacteria obtain their energy through photosynthesis, it has been proposed that the cyanobacterial clock must then sense cellular redox state. Application of the redox-active quinone group of compounds to the Kai proteins *in vitro* has been shown to induce phase shifts, and can also serve as a synchronising zeitgeber to protein mixtures that are in different phases (Kim et al., 2012) by binding to KaiA, resulting in its aggregation (Wood et al., 2010). Although the detailed mechanisms of the entrainment of the reconstituted *in vitro* oscillator by the oxidation state of quinone remain unclear, it has been known for at least three decades that the cellular redox states of plants changes over circadian time (Wagner et al., 1975). Through many chronobiologists have long assumed that metabolic rhythms are a functional *readout* of the circadian clock, the activity of the quinones in cyanobacteria suggests that redox states likely play an integral role in circadian timing.

Variants of the Cryptochrome protein (CRY) in *Arabidopsis*, *Drosophila*, and mouse provide the most direct path by which redox status can interact with the core components of the TTFL. In insects and plants, CRY is directly photosensitive, functioning as a circadian-dependent blue-light photoreceptor (Emery et al., 1998, 2000; Egan et al., 1999). CRY shares homology with a phylogenetically ancient enzyme family that repairs DNA in response to ultraviolent light known as the photolyases, leading to speculation that redox is therefore crucial to CRY function (Selby and Sancar, 2006; Müller and Carell, 2009). Indeed, the CRY protein does contain motifs that bind the flavins (Emery et al., 1998; Müller and Carell, 2009; Stanewsky et al., 1998), a group of organic compounds known for their prominent role in electron transport in many metabolic reactions and also their ability to be reduced by the incidence of light.

Blocking electron transport was first demonstrated to attenuate the degradation of *Drosophila* CRY (dCRY) (Lin et al., 2001). Later, it was discovered that purified dCRY binds to oxidised flavin adenine dinucleotide (FAD), which is then reduced to the semiquinone radical $FAD^{\bullet -}$ under blue-light illumination (Berndt et al., 2007). This event results in the activation of CRY by allowing it to bind to its partner TIMELESS (Vaidya et al., 2013) as part of the fly TTFL. Mutagenesis of the FAD binding site in CRY residues abolishes its responses to light in *Drosophila* S2 cells (Froy et al., 2002). Two groups have attempted to solve the crystal structure of *Drosophila* CRY, although there are significant differences between the two proposed structures that mediate the reduction of FAD (Zoltowski et al., 2011; Czarna et al., 2013). Mutations of the corresponding FAD-binding residues in mouse CRY (mCRY) do not abolish the inhibitory function it has on transcription (Froy et al., 2002), implying that the function of mCRY is unlikely to be redox dependent. Structural analysis of mCRY reveals that while it possesses the ability to bind FAD, it is likely involved in interactions with other proteins such as the F-box protein FBXL3, an E3 ubiquitin ligase, which binds across the FAD binding pocket (Czarna et al., 2013). Along with FBXL21, these proteins regulate mCRY degradation and the period of the circadian clock (Dardente et al., 2008; Hirano et al., 2013; Busino et al., 2007).

Nicotinamide adenine dinucleotide (NAD) coenzymes are involved in a variety of metabolic oxidation—reduction reactions with the major source of the reducing agent NADPH in non-photosynthetic organisms being generated by the pentose phosphate pathway. It has also been shown that NADs are capable of modulating the DNA binding of circadian heterodimers such as CLOCK:BMAL1 and NPAS2:BMAL1. In biochemical assays performed *in vitro*, the reducing factors NADH and NADPH, have been shown to promote DNA binding, whereas the oxidised forms, NAD and NADP, were inhibitory (Rutter et al., 2001). Studies have demonstrated that the first 61 N-terminal amino acids of NPAS2 are sufficient to sense NADPH (Yoshii et al., 2013), and that the increase of DNA-binding activity with increasing pH is also enhanced by NADPH addition (Yoshii et al., 2015). These results suggest that the activity of some components of the TTFL, such as NPAS2, activity can also be modulated by its function as a metabolic sensor. The results of these studies demonstrate that redox mechanisms are capable of regulating existing machinery already recognised as part of the circadian clockwork, but the question remains of whether or not cells or organisms without transcriptional mechanisms — *i.e.*, not possessing a TTFL — also possess a circadian pacemaker.

Redox the clock: keeping time without transcription and translation

Red blood cells are anucleate and lack both transcriptional and translational mechanisms. Because they can be readily and easily harvested from wide variety of mammals and humans, they provide a unique cellular model for answering some fundamental questions about the basis of the molecular clock: without a TTFL can cells like these still keep time? Surprisingly, the answer is ''yes''. The first observation of a TTFL-independent mechanism of timekeeping was performed by O'Neill and Reddy: they discovered that the over- and hyperoxidised forms of peroxiredoxin (Prdx) exhibited a clear circadian pattern in human red blood cells (O'Neill and Reddy, 2011). These oscillations were found to have a period of approximately 24 h,

and demonstrated temperature compensation (O'Neill and Reddy, 2011) — a phenomenon where the periodicity does not change between temperature ranges of 32 and 37 °C, long considered one of the hallmark characteristics of a true circadian rhythm (Pittendrigh, 1960). Furthermore, this rhythm is entrainable by temperature, so that the peaks and troughs of Prdx overoxidation coincide with low (36.8 °C) and high (37.4 °C) temperatures, respectively (O'Neill and Reddy, 2011). These observations fulfil some requirements for the Prdx rhythms to be considered an independent timekeeper on its own, and not merely a functional readout of the TTFL.

The peroxiredoxins are a family of antioxidant molecules that modulate intracellular levels of hydrogen peroxide. They are believed to be an evolutionarily ancient mechanism, as they are highly conserved throughout eukaryotes, prokaryotes and even archaea (Edgar et al., 2012). It is thought that they developed from a thioredoxin-like precursor (Copley et al., 2004). Peroxiredoxins are grouped as 1-Cys or 2-Cys families, depending on the how the catalytic cysteines are provided. In the 1-Cys variety, intramolecular disulphides are formed, whereas in the 2-Cys family, an intermolecular bond is formed, resulting in dimer formation.

All activity performed by the peroxiredoxins can be summarised into three main processes: peroxidation, resolution, and recycling. Peroxidation is the reduction of peroxide through the oxidation of a conserved 'peroxidatic' cysteine residue (C_P), which results in the formation of a sulphenic acid (C_P-SOH) within the enzyme's active site. In the 2-Cys group, the resolution step occurs with the release of a water molecule when C_P forms a disulphide bond with a second, free thiol group (C_R, for resolving cysteine) near the C-terminus of a partner molecule. Recycling is the third and final step of the catalytic cycle, wherein the disulphide bond is broken by a thioredoxin-like molecule and C_P and C_R are returned to their free thiol state (Hall et al., 2009). In some systems, another branch of the catalytic reaction exists, where C_P can undergo over-oxidation to the sulphinic (C_P-SO_2H) form, or even hyper-oxidation into a sulphonic (CP-SO_3H) state. The over-oxidised sulphinic form can be reduced by sulphiredoxin (Srx) in an ATP-dependent reaction that is part of normal redox regulation and repair mechanisms (Hall et al., 2009), however the whilst hyper-oxidised sulphonic acid form is irreversible, and can therefore potentially serve as a marker of cumulative oxidative stress (Lim et al., 2008).

Recent studies have confirmed the existence of Prdx oscillations in mouse red blood cells both wild type and mutant animals lacking *Srx* (Cho et al., 2014). Surprisingly, the rhythmicity of Prdx over-oxidation in *Srx* knockout mice was unchanged (Cho et al., 2014), despite its involvement in the reduction of the sulphinic form. Perturbation of the redox balance in red blood cells, however, can interfere with the redox oscillator: Prdx rhythms are abolished in SOD1-deficient mice, and the Prdx2 protein exhibit higher levels of over- and hyper-oxidation, since SOD1 is the sole scavenger of the superoxide radical in red blood cells (Homma et al., 2015). Behavioural recordings of these mutants revealed no differences in the periodicity of activity (Homma et al., 2015), so the question remains as to how the redox oscillation integrates with the organism at the behavioural level.

Mammalian RBCs are not the only system in which the oscillation of Prdxs have been observed, they have also been found in *Ostreococcus tauri* (O'Neill et al., 2011), the smallest known alga. This organism shares some homology with the *Arabidopsis* plant, namely the oscillatory expression of the plant clock genes *TOC1* and *CCA1* genes (Corellou et al., 2009). The same circadian pattern of the over- and hyper-oxidised forms of Prdx were also found in *O. tauri*, and it was found that these rhythms persisted even when the cells were transferred into total darkness, when the absence of light in these phototrophic organisms shut down gene transcription. Theoretically, if circadian rhythms were entirely dependent on the cyclic expression of the genes that make up the TTFL, transferring these cells back into a light environment should result in a resetting of the circadian clock's phase. This, however, was not observed, and instead the *O. tauri* exhibited a phase-dependent response to re-illumination, meaning that a non-transcriptional mechanism had been responsible for keeping time whilst the cells were in the dark, without *de novo* transcription (O'Neill et al., 2011). These observations demonstrated for the first time that redox mechanisms, in the form of Prdx oxidation cycles, were able to reflect circadian timekeeping in the absence of transcriptional mechanisms in eukaryotic systems that ordinarily relied up on their TTFL.

Rhythms of Prdx oxidation can be coupled to the TTFL, since long-period mutants of *O. tauri* exhibited similarly lengthened periodicity of gene expression and Prdx activity (O'Neill et al., 2011). Prdxs are so ubiquitous throughout nature that circadian oscillations in their over- and hyper-oxidised forms are found in the archaeon *Halobacterium salinarum*, the cyanobacterium *Synechococcus*, the yeast strain *Saccharomyces cerevisae* (Causton et al., 2015), the fungus *Neurospora crassa*, *Drosophila*, and all the way up to mice and humans (Edgar et al., 2012). Circadian patterns in the nuclear and cytoplasmic translocation of Prdx2 have been described in immortalised HaCaT human keratinocytes after synchronisation with temperature cycles (Avitabile et al., 2014), and the phase of Prdx oxidation is tied directly, in yeast, to its respiratory cycles (Causton et al., 2015). Because of the highly conserved nature of the Prdx oscillator, we can entertain the possibility redox reactions and cellular metabolism might be more than a readout or redundant backup system secondary to the TTFL. Perhaps redox plays a more central role in circadian rhythms, the integral key, common amongst all the phyla which unites the timing of life to the revolution of the earth, and the resultant rising and setting of the sun.

Further links between redox metabolism and the circadian clock

Circadian rhythms in the generation and scavenging of ROS have been observed in a variety of species, from plants (Lai et al., 2012) to fungi (Yoshida et al., 2011), worms (Olmedo et al., 2012), flies (Beaver et al., 2012; Krishnan et al., 2008), and rodents (Wang et al., 2012). The involvement

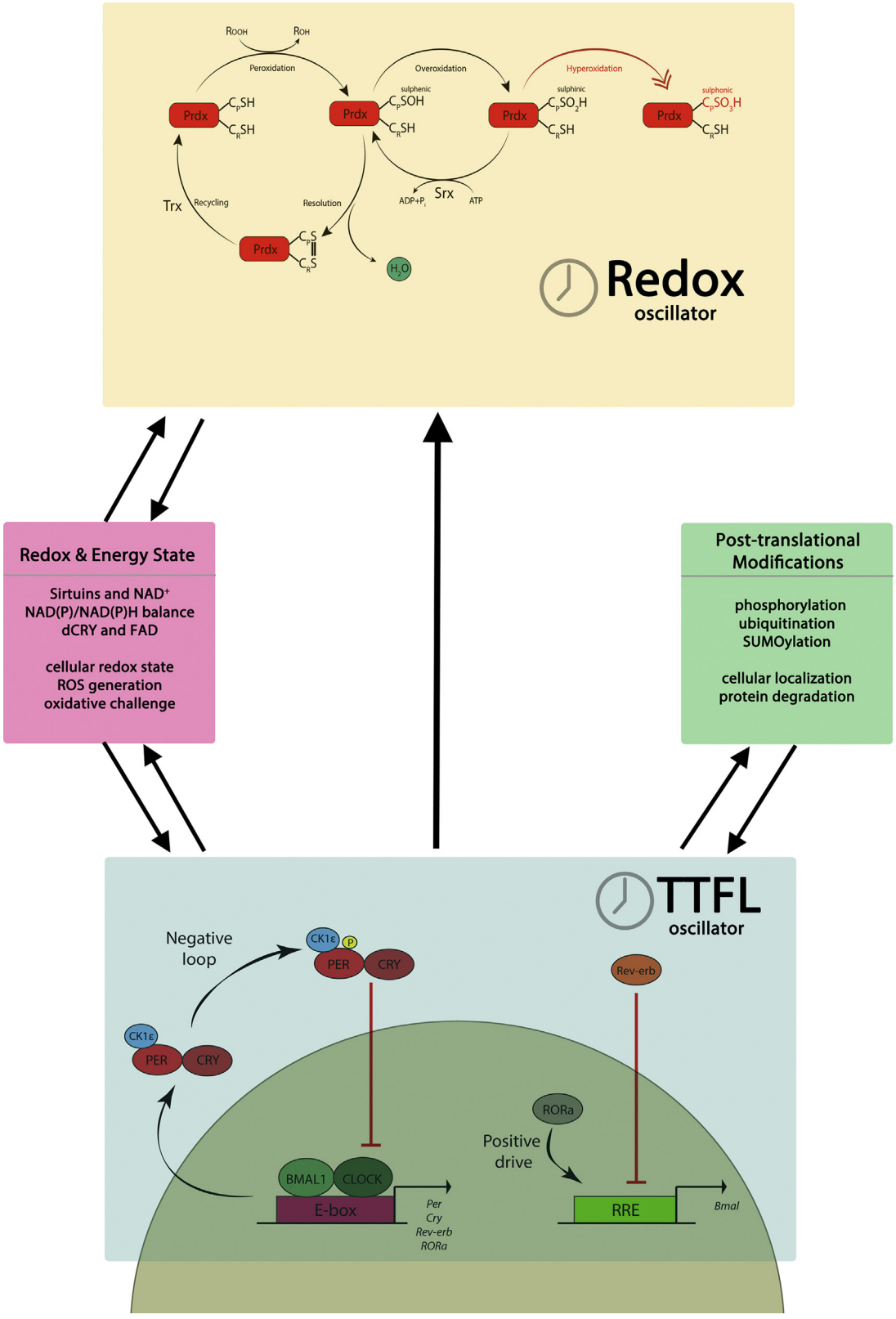

Figure 1 A simplified schematic depicting the known interactions between the molecular pacemaker, the redox oscillator, and other regulatory mechanisms. The transcription–translation feedback loop (TTFL) model describes circadian rhythms in gene expression of the core clock genes, is the most well-known mechanism of cellular timekeeping. It can be modified by post-translational modifications such as phosphorylation and ubiquitination, which regulate cellular localisation and proteasomal degradation. It is now known that the redox and energy state of the cell can also play a role in regulating the TTFL, as reciprocal relationships exist through direct interaction with TTFL components such as CRYPTOCHROME or NPAS2 (Neuronal PAS Domain Protein 2). However, the

of the clockwork in the regulation of cellular redox state is by studies where deletion or disruption mutations of the clock genes also result in the perturbation of these systems. In the fungus *Neurospora crassa*, arrhythmic mutants for the clock genes *white collar-1* (Δ*wc-1*) and *white collar-2* (Δ*wc-2*) have their rhythmic expression of the catalase gene, *cat-1*, abolished (Yoshida et al., 2011). Underscoring the interplay between redox and the clock genes, it has also been observed that mutations in the *sod-1* gene result in a more robust and stable circadian rhythm of conidiation banding — asexual spore formation — when compared with wild type fungi. Generation of ROS such as the superoxide radical and H_2O_2 also follow a circadian pattern, and regulate the transcriptional function of the WHITE COLLAR protein (Yoshida et al., 2011).

These interactions between redox state and the TTFL oscillator are also further underscored in experiments in cyanobacteria and *Arabidopsis* deficient in 2-Cys Prdxs, these organisms still exhibit circadian rhythms, but they are different from wild type oscillations in either phase or amplitude (Edgar et al., 2012). Conversely, the two *Drosophila* mutant lines, Clk^{Jrk} and per^{01} are behaviourally arrhythmic, but still exhibit cycles of Prdx oxidation, again with altered parameters compared to their wild type counterparts. Thus, perturbation of the TTFL clockwork or the redox system results in a perturbation of the other, indicating that they have a reciprocal relationship. This also implies that metabolic mechanisms deserve greater scrutiny in the context of circadian rhythms, as the two systems may be more integrated than previously thought (Rutter et al., 2002; van Ooijen and Millar, 2012; Merrow and Roenneberg, 2001).

The effects of oxidative stress and oxidative challenges may be processes that are mediated by the circadian clock. Addition of exogenous H_2O_2, a potent oxidiser and one of the key players in oxidative cell damage, to experimental cultures of *Microcystis aeruginosa* cyanobacteria have been demonstrated to alter the diurnal expression of the Kai oscillator through induced phase-shifts. Susceptibility to oxidative stress through disruption of the circadian oscillators is therefore a proposed mechanism by which hydrogen peroxide can act as an effective chemical algaecide (Qian et al., 2010).

Drosophila exhibit similar properties, in that there is a circadian component to the fly's susceptibility to oxidative stress. Wild type Canton-S flies experience lower mortality rates when exposed to exogenous H_2O_2 in the night time compared to the flies subjected to an environment of constant light, which disrupts the circadian clockwork. Per^{01} flies with genetically disrupted circadian behaviour were more susceptible to oxidative challenge overall. These observations coincided with increased endogenous mitochondrial production of H_2O_2, as well as enhanced protein carbonylation of catalase (Krishnan et al., 2008). Comparable results have emerged in clock mutant models in *Arabidopsis*, where plants with a mutation in the clock gene *CCA1* have decreased catalase activity, and are more sensitive to ROS generating agents. The overexpression of *CCA1* results in the suppression of H_2O_2 levels (Lai et al., 2012). Similar results can be found in mammalian models *in vitro*, where studies have demonstrated that mouse embryonic fibroblasts deficient in *Bmal1* have lower survival rates when undergoing a highly concentrated, near-lethal application of H_2O_2 (Tamaru et al., 2013). The same study also found that such a critical event of oxidative stress resets the circadian clock in other mammalian cell lines, resulting in a concurrent activation of a network of circadian genes that then continued onward to modulate an antioxidant, cell survival response (Tamaru et al., 2013). These results strongly suggest that the circadian clockwork is involved in complex cellular programmes that regulate endogenous ROS and also defend the organism against exogenous oxidative challenge. Current evidence seems to support the conclusion that the responses to ROS are mediated both through the regular function of the molecular clockwork and the involvement of the TTFL genes in extra-circadian pathways.

In addition to the role that hydrogen peroxide plays in oxidative damage, it has also been recognised as an intracellular messenger in oxidation-dependent steps in signal transduction (Stone and Yang, 2006). Studies in cultured zebrafish Z3 cells, a cell line derived from embryonic fish, have demonstrated that light induces the production of H_2O_2, which results in an activation of the expression of the zebrafish TTFL genes, *zCry1a* and *zPer2*. This suggests that hydrogen peroxide may act as a signal transducer, relaying information about the light environment to the circadian pacemaker (Hirayama et al., 2007b). This function should not be surprising one, as the clocks in zebrafish peripheral tissues are directly light-sensitive (Tamai et al., 2005). Catalase activity in this zebrafish cell line also appears to inhibit the expression of the *zCry1a* and *zPer2* genes through the reduction of the amount of H_2O_2 in the cell (Hirayama et al., 2007b). These observations provide a link by which ROS may regulate the expression of the circadian clock.

Conclusion and perspectives

From the large array of experimental evidence available, we now know that various redox mechanisms and the metabolic state of the cell can interact with the circadian machinery (Fig. 1). These vary from phosphorylation and other post-translational modifications, to the circadian oscillation of redox responses such as the Prdx proteins, which are able to function as circadian pacemakers when the TTFL cannot. The plethora of redox and metabolic pathways have yet to point to a single, coherent mechanism by which reduction and oxidation may govern circadian rhythms, but we know

recently elucidated redox oscillator can function as a pacemaker in the absence of transcription in model systems such as the alga *Ostreococcus tauri*, and the periodicity of the peroxiredoxin oscillator can be determined by mutants with aberrant TTFL periodicity. Empty spaces represent areas of future research, especially where we hope to soon discover the mechanisms by which the redox oscillator can affect the TTFL.

from nature as a whole that answers like this are rarely simple and clear cut. Nevertheless, the common narrative that emerges from these studies is that cellular metabolism does interact with an organism's clock, and the reciprocal relationship between metabolic and circadian systems indicates that both play an important role in maintaining the function of the organism as it proceeds through the ebb and flow of time.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

ABR is a Wellcome Trust Senior Clinical Fellow. He receives funding from the Wellcome Trust (grant no. 100333/Z/12/Z), the European Research Council (ERC Starting grant no. 281348, MetaCLOCK), the European Molecular Biology Organization (EMBO) Young Investigators Programme, and the Lister Institute of Preventative Medicine. LW is supported by the European Research Council.

References

Akten, B., Jauch, E., Genova, G.K., Kim, E.Y., Edery, I., Raabe, T., Jackson, F.R., 2003. A role for CK2 in the Drosophila circadian oscillator. Nat. Neurosci. 6, 251—257, http://dx.doi.org/10.1038/nn1007.

Asher, G., Gatfield, D., Stratmann, M., Reinke, H., Dibner, C., Kreppel, F., Mostoslasky, R., Alt, F.W., Schibler, U., 2008. SIRT1 regulates circadian clock gene expression through PER2 deacetylation. Cell 134, 317—328, http://dx.doi.org/10.1016/j.cell.2008.06.050.

Avitabile, D., Ranieri, D., Nicolussi, A., D'Inzeo, S., Capriotti, A.L., Genovese, L., Proietti, S., Cucina, A., Coppa, A., Samperi, R., Bizzarri, M., Laganà, A., Torrisi, M.R., 2014. Peroxiredoxin 2 nuclear levels are regulated by circadian clock synchronization in human keratinocytes. Int. J. Biochem. Cell Biol. 53, 24—34, http://dx.doi.org/10.1016/j.biocel.2014.04.024.

Baydas, G., Gursu, M.F., Yilmaz, S., Canpolat, S., Yasar, A., Cikim, G., Canatan, H., 2002. Daily rhythm of glutathione peroxidase activity, lipid peroxidation and glutathione levels in tissues of pinealectomized rats. Neurosci. Lett. 323, 195—198, http://dx.doi.org/10.1016/s0304-3940(02)00144-1.

Beaver, L.M., Klichko, V.I., Chow, E.S., Kotwica-Rolinska, J., Williamson, M., Orr, W.C., Radyuk, S.N., Giebultowicz, J.M., 2012. Circadian regulation of glutathione levels and biosynthesis in *Drosophila melanogaster*. PLoS ONE 7, e50454, http://dx.doi.org/10.1371/journal.pone.0050454.

Berndt, A., Kottke, T., Breitkreuz, H., Dvorsky, R., Hennig, S., Alexander, M., Wolf, E., 2007. A novel photoreaction mechanism for the circadian blue light photoreceptor *Drosophila* cryptochrome. J. Biol. Chem. 282, 13011—13021, http://dx.doi.org/10.1074/jbc.m608872200.

Busino, L., Bassermann, F., Maiolica, A., Lee, C., Nolan, P.M., Godinho, S.I.H., Draetta, G.F., Pagano, M., 2007. SCFFbxl3 controls the oscillation of the circadian clock by directing the degradation of cryptochrome proteins. Science 316, 900—904, http://dx.doi.org/10.1126/science.1141194.

Cardone, L., Hirayama, J., Giordano, F., Tamaru, T., Palvimo, J.J., Sassone-Corsi, P., 2005. Circadian clock control by SUMOylation of BMAL1. Science 309, 1390—1394, http://dx.doi.org/10.1126/science.1110689.

Causton, H.C., Feeney, K.A., Ziegler, C.A., O'Neill, J.S., 2015. Metabolic cycles in yeast share features conserved among circadian rhythms. Curr. Biol. 25, 1056—1062, http://dx.doi.org/10.1016/j.cub.2015.02.035.

Cho, C.-S., Yoon, H.J., Kim, J.Y., Woo, H.A., Rhee, S.G., 2014. Circadian rhythm of hyperoxidized peroxiredoxin II is determined by hemoglobin autoxidation and the 20S proteasome in red blood cells. Proc. Natl. Acad. Sci. U.S.A. 111, 12043—12048, http://dx.doi.org/10.1073/pnas.1401100111.

Copley, S.D., Novak, W.R.P., Babbitt, P.C., 2004. Divergence of function in the thioredoxin fold suprafamily: evidence for evolution of peroxiredoxins from a thioredoxin-like ancestor. Biochemistry 43, 13981—13995, http://dx.doi.org/10.1021/bi048947r.

Corellou, F., Schwartz, C., Motta, J.-P., Djouani-Tahri, E.B., Sanchez, F., Bouget, F.-Y., 2009. Clocks in the green lineage: comparative functional analysis of the circadian architecture of the picoeukaryote *Ostreococcus*. Plant Cell 21, 3436—3449, http://dx.doi.org/10.1105/tpc.109.068825.

Czarna, A., Berndt, A., Singh, H.R., Grudziecki, A., Ladurner, A.G., Timinszky, G., Kramer, A., Wolf, E., 2013. Structures of *Drosophila* cryptochrome and mouse cryptochrome1 provide insight into circadian function. Cell 153, 1394—1405, http://dx.doi.org/10.1016/j.cell.2013.05.011.

Dardente, H., Mendoza, J., Fustin, J.-M., Challet, E., Hazlerigg, D.G., 2008. Implication of the F-Box Protein FBXL21 in circadian pacemaker function in mammals. PLoS ONE 3, e3530, http://dx.doi.org/10.1371/journal.pone.0003530.

Doi, M., Hirayama, J., Sassone-Corsi, P., 2006. Circadian regulator CLOCK is a histone acetyltransferase. Cell 125, 497—508, http://dx.doi.org/10.1016/j.cell.2006.03.033.

Edgar, R.S., Green, E.W., Zhao, Y., van Ooijen, G., Olmedo, M., Qin, X., Xu, Y., Pan, M., Valekunja, U.K., Feeney, K.A., Maywood, E.S., Hastings, M.H., Baliga, N.S., Merrow, M., Millar, A.J., Johnson, C.H., Kyriacou, C.P., O'Neill, J.S., Reddy, A.B., 2012. Peroxiredoxins are conserved markers of circadian rhythms. Nature 485, 459—464, http://dx.doi.org/10.1038/nature11088.

Egan, E.S., Franklin, T.M., Hilderbrand-Chae, M.J., McNeil, G.P., Roberts, M.A., Schroeder, A.J., Zhang, X., Jackson, F.R., 1999. An extraretinally expressed insect cryptochrome with similarity to the blue light photoreceptors of mammals and plants. J. Neurosci. 19, 3665—3673.

Emery, P., So, W.V., Kaneko, M., Hall, J.C., Rosbash, M., 1998. CRY, a *Drosophila* clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity. Cell 95, 669—679, http://dx.doi.org/10.1016/s0092-8674(00)81637-2.

Emery, P., Stanewsky, R., Helfrich-Förster, C., Emery-Le, M., Hall, J.C., Rosbash, M., 2000. *Drosophila* CRY is a deep brain circadian photoreceptor. Neuron 26, 493—504, http://dx.doi.org/10.1016/s0896-6273(00)81181-2.

Filomeni, G., Rotilio, G., Ciriolo, M.R., 2003. Glutathione disulfide induces apoptosis in U937 cells by a redox-mediated p38 MAP kinase pathway. FASEB J. 17, 64—66, http://dx.doi.org/10.1096/fj.02-0105fje.

Froy, O., Chang, D.C., Reppert, S.M., 2002. Redox potential: differential roles in dCRY and mCRY1 functions. Curr. Biol. 12, 147—152, http://dx.doi.org/10.1016/s0960-9822(01)00656-x.

Gardner, M.J., Hubbard, K.E., Hotta, C.T., Dodd, A.N., Webb, A.A.R., 2006. How plants tell the time. Biochem. J. 397, 15—24, http://dx.doi.org/10.1042/bj20060484.

Hall, A., Karplus, P.A., Poole, L.B., 2009. Typical 2-Cys peroxiredoxins — structures, mechanisms and functions. FEBS J. 276, 2469—2477, http://dx.doi.org/10.1111/j.1742-4658.2009.06985.x.

Hansen, J., 2001. Increased breast cancer risk among women who work predominantly at night. Epidemiology 12, 74—77, http://dx.doi.org/10.1097/00001648-200101000-00013.

Hansen, J., Stevens, R.G., 2012. Case—control study of shift-work and breast cancer risk in Danish nurses: impact of shift systems. Eur. J. Cancer 48, 1722—1729, http://dx.doi.org/10.1016/j.ejca.2011.07.005.

Hardeland, R., Coto-Montes, A., Poeggeler, B., 2003. Circadian rhythms, oxidative stress, and antioxidative defense mechanisms. Chronobiol. Int. 20, 921—962, http://dx.doi.org/10.1081/cbi-120025245.

Hardin, P.E., Hall, J.C., Rosbash, M., 1990. Feedback of the *Drosophila* period gene product on circadian cycling of its messenger RNA levels. Nature 343, 536—540, http://dx.doi.org/10.1038/343536a0.

Hastings, M.H., Reddy, A.B., Maywood, E.S., 2003. A clockwork web: circadian timing in brain and periphery, in health and disease. Nat. Rev. Neurosci. 4, 649—661, http://dx.doi.org/10.1038/nrn1177.

Healy, D., Minors, D.S., Waterhouse, J.M., 1993. Shiftwork, helplessness and depression. J. Affect. Disorders 29, 17—25, http://dx.doi.org/10.1016/0165-0327(93)90114-y.

Hirano, A., Yumimoto, K., Tsunematsu, R., Matsumoto, M., Oyama, M., Kozuka-Hata, H., Nakagawa, T., Lanjakornsiripan, D., Nakayama, K.I., Fukada, Y., 2013. FBXL21 regulates oscillation of the circadian clock through ubiquitination and stabilization of cryptochromes. Cell 152, 1106—1118, http://dx.doi.org/10.1016/j.cell.2013.01.054.

Hirayama, J., Sahar, S., Grimaldi, B., Tamaru, T., Takamatsu, K., Nakahata, Y., Sassone-Corsi, P., 2007a. CLOCK-mediated acetylation of BMAL1 controls circadian function. Nature 450 (7172), 1086—1090, http://dx.doi.org/10.1038/nature06394.

Hirayama, J., Cho, S., Sassone-Corsi, P., 2007b. Circadian control by the reduction/oxidation pathway: catalase represses light-dependent clock gene expression in the zebrafish. Proc. Natl. Acad. Sci. U.S.A. 104, 15747—15752, http://dx.doi.org/10.1073/pnas.0705614104.

Homma, T., Okano, S., Lee, J., Ito, J., Otsuki, N., Kurahashi, T., Kang, E.S., Nakajima, O., Fujii, J., 2015. SOD1 deficiency induces the systemic hyperoxidation of peroxiredoxin in the mouse. Biochem. Biophys. Res. Commun. 463, 1040—1046, http://dx.doi.org/10.1016/j.bbrc.2015.06.055.

Huang, T.C., Tu, J., Chow, T.J., Chen, T.H., 1990. Circadian rhythm of the prokaryote *Synechococcus* sp. RF-1. Plant Physiol. 92, 531—533, http://dx.doi.org/10.1104/pp.92.2.531.

Huang, W., Ramsey, K.M., Marcheva, B., Bass, J., 2011. Review series: Circadian rhythms, sleep, and metabolism. J. Clin. Investig. 121, 2133—2141, http://dx.doi.org/10.1172/jci46043.

Ishiura, M., Kutsuna, S., Aoki, S., Iwasaki, H., Andersson, C.R., Tanabe, A., Goldon, S.S., Johnson, C.H., Kondo, T., 1998. Expression of a gene cluster kaiABC as a circadian feedback process in cyanobacteria. Science 281, 1519—1523, http://dx.doi.org/10.1126/science.281.5382.1519.

Janszky, I., Ljung, R., 2008. Shifts to and from daylight saving time and incidence of myocardial infarction. N. Engl. J. Med. 359, 1966—1968, http://dx.doi.org/10.1056/nejmc0807104.

Janszky, I., Ahnve, S., Ljung, R., Mukamal, K.J., Gautam, S., Wallentin, L., Stenestrand, U., 2012. Daylight saving time shifts and incidence of acute myocardial infarction — Swedish Register of Information and Knowledge About Swedish Heart Intensive Care Admissions (RIKS-HIA). Sleep Med. 13, 237—242, http://dx.doi.org/10.1016/j.sleep.2011.07.019.

Keesler, G.A., Camacho, F., Guo, Y., Virshup, D., Mondadori, C., Yao, Z., 2000. Phosphorylation and destabilization of human period I clock protein by human casein kinase I epsilon. Neuroreport 11, 951—955, http://dx.doi.org/10.1097/00001756-200004070-00011.

Kim, Y.-I., Vinyard, D.J., Ananyev, G.M., Dismukes, G.C., Golden, S.S., 2012. Oxidized quinones signal onset of darkness directly to the cyanobacterial circadian oscillator. Proc. Natl. Acad. Sci. U.S.A. 109, 17765—17769, http://dx.doi.org/10.1073/pnas.1216401109.

Kondo, T., Ishiura, M., 2000. The circadian clock of cyanobacteria. Bioessays 22, 10—15, http://dx.doi.org/10.1002/(sici)1521-1878(200001)22:1<10::aid-bies4>3.0.co;2-a.

Konopka, R.J., Benzer, S., 1971. Clock mutants of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S.A. 68, 2112—2116, http://dx.doi.org/10.1073/pnas.68.9.2112.

Krishnan, N., Davis, A.J., Giebultowicz, J.M., 2008. Circadian regulation of response to oxidative stress in *Drosophila melanogaster*. Biochem. Biophys. Res. Commun. 374, 299—303, http://dx.doi.org/10.1016/j.bbrc.2008.07.011.

Lai, A.G., Doherty, C.J., Mueller-Roeber, B., Kay, S.A., Schippers, J.H.M., Dijkwel, P.P., 2012. CIRCADIAN CLOCK-ASSOCIATED 1 regulates ROS homeostasis and oxidative stress responses. Proc. Natl. Acad. Sci. U.S.A. 109, 17129—17134, http://dx.doi.org/10.1073/pnas.1209148109.

Lee, J., Lee, Y., Lee, M.J., Park, E., Kang, S.H., Chung, C.H., Lee, K.K., 2008. Dual modification of BMAL1 by SUMO2/3 and ubiquitin promotes circadian activation of the CLOCK/BMAL1 complex. Mol. Cell. Biol. 28, 6056—6065, http://dx.doi.org/10.1128/mcb.00583-08.

Lim, J.C., Choi, H.-I., Park, Y.S., Nam, H.W., Woo, H.A., Kwon, K.-S., Kim, S.G., Rhee, S.G., Kim, K., Chae, H.Z., 2008. Irreversible oxidation of the active-site cysteine of peroxiredoxin to cysteine sulfonic acid for enhanced molecular chaperone activity. J. Biol. Chem. 283, 28873—28880, http://dx.doi.org/10.1074/jbc.m804087200.

Lin, F.J., Song, W., Meyer-Bernstein, E., Naidoo, N., Sehgal, A., 2001. Photic signaling by cryptochrome in the *Drosophila* circadian system. Mol. Cell. Biol. 21, 7287—7294, http://dx.doi.org/10.1128/mcb.21.21.7287-7294.2001.

Lowrey, P.L., Shimomura, K., Antoch, M.P., Yamazaki, S., Zemenides, P.D., Ralph, M.R., Menaker, M., Takahashi, J.S., 2000. Positional syntenic cloning and functional characterization of the mammalian circadian mutation tau. Science 288, 483—492, http://dx.doi.org/10.1126/science.288.5465.483.

Mehra, A., Baker, C.L., Loros, J.J., Dunlap, J.C., 2009. Post-translational modifications in circadian rhythms. Trends Biochem. Sci. 34, 483—490, http://dx.doi.org/10.1016/j.tibs.2009.06.006.

Meng, Q.J., Logunova, L., Maywood, E.S., Gallego, M., Lebiecki, J., Brown, T.M., Sládek, M., Semikhodskiim, A.S., Glossop, N.R.J., Piggins, H.D., Chesham, J.E., Bechtold, D.A., Yoo, S.H., Takahashi, J.S., Virshup, D.M., Boot-Handford, R.P., Hastings, M.H., Loudon, A.S.I., 2008. Setting clock speed in mammals: the CK1ε tau mutation in mice accelerates circadian pacemakers by selectively destabilizing PERIOD proteins. Neuron 58, 78—88, http://dx.doi.org/10.1016/j.neuron.2008.01.019.

Merrow, M., Roenneberg, T., 2001. Circadian clocks: running on redox. Cell 106, 141—143, http://dx.doi.org/10.1016/s0092-8674(01)00443-3.

Mitsui, A., Kumazawa, S., Takahashi, A., Ikemoto, H., Cao, S., Arai, T., 1986. Strategy by which nitrogen-fixing unicellular cyanobacteria grow photoautotrophically. Nature 323, 720—722, http://dx.doi.org/10.1038/323720a0.

Müller, M., Carell, T., 2009. Structural biology of DNA photolyases and cryptochromes. Curr. Opin. Struct. Biol. 19, 277—285, http://dx.doi.org/10.1016/j.sbi.2009.05.003.

Nakahata, Y., Kaluzova, M., Grimaldi, B., Sahar, S., Hirayama, J., Chen, D., Guarente, L.P., Sassone-Corsi, P., 2008. The NAD+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodelling and circadian control. Cell 134, 329—340, http://dx.doi.org/10.1016/j.cell.2008.07.002.

Nakajima, M., Imai, K., Ito, H., Nishiwaki, T., Murayama, Y., Iwasaki, H., Oyama, T., Kondo, T., 2005. Reconstitution of circadian oscillation of cyanobacterial KaiC phosphorylation *in vitro*. Science 308, 414—415, http://dx.doi.org/10.1126/science.1108451.

Olmedo, M., O'Neill, J.S., Edgar, R.S., Valekunja, U.K., Reddy, A.B., Merrow, M., 2012. Circadian regulation of olfaction and an evolutionarily conserved, nontranscriptional marker in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. U.S.A. 109, 20479—20484, http://dx.doi.org/10.1073/pnas.1211705109.

O'Neill, J.S., Reddy, A.B., 2011. Circadian clocks in human red blood cells. Nature 469, 498—503, http://dx.doi.org/10.1038/nature09702.

O'Neill, J.S., van Ooijen, G., Dixon, L.E., Troein, C., Corellou, F., Bouget, F.-Y., Reddy, A.K., Millar, A.J., 2011. Circadian rhythms persist without transcription in a eukaryote. Nature 469, 554—558, http://dx.doi.org/10.1038/nature09654.

Park, H.-A., Khanna, S., Rink, C., Gnyawali, S., Roy, S., Sen, C.K., 2009. Glutathione disulfide induces neural cell death via a 12-lipoxygenase pathway. Cell Death Differ. 16, 1167—1179, http://dx.doi.org/10.1038/cdd.2009.37.

Pittendrigh, C.S., 1960. Circadian rhythms and the circadian organization of living systems. Cold Spring Harb. Symp. Quant. Biol. 25, 159—184, http://dx.doi.org/10.1101/sqb.1960.025.01.015.

Qian, H., Yu, S., Sun, Z., Xie, X., Liu, W., Fu, Z., 2010. Effects of copper sulfate, hydrogen peroxide and N-phenyl-2-naphthylamine on oxidative stress and the expression of genes involved photosynthesis and microcystin disposition in *Microcystis aeruginosa*. Aquat. Toxicol. 99, 405—412, http://dx.doi.org/10.1016/j.aquatox.2010.05.018.

Reddy, P., Zehring, W.A., Wheeler, D.A., Pirrotta, V., Hadfield, C., Hall, J.C., Rosbash, M., 1984. Molecular analysis of the period locus in *Drosophila melanogaster* and identification of a transcript involved in biological rhythms. Cell 38, 701—710, http://dx.doi.org/10.1016/0092-8674(84)90265-4.

Reppert, S.M., Weaver, D.R., 2002. Coordination of circadian timing in mammals. Nature 418, 935—941, http://dx.doi.org/10.1038/nature00965.

Roenneberg, T., Merrow, M., 2005. Circadian clocks — the fall and rise of physiology. Nat. Rev. Mol. Cell Biol. 6, 965—971, http://dx.doi.org/10.1038/nrm1766.

Rutter, J., Reick, M., Wu, L.C., McKnight, S.L., 2001. Regulation of clock and NPAS2 DNA binding by the redox state of NAD cofactors. Science 293, 510—514, http://dx.doi.org/10.1126/science.1060698.

Rutter, J., Reick, M., McKnight, S.L., 2002. Metabolism and the control of circadian rhythms. Annu. Rev. Biochem. 71, 307—331, http://dx.doi.org/10.1146/annurev.biochem.71.090501.142857.

Sathyanarayanan, S., Zheng, X., Xiao, R., Sehgal, A., 2004. Posttranslational regulation of *Drosophila* PERIOD protein by protein phosphatase 2A. Cell 116, 603—615, http://dx.doi.org/10.1016/s0092-8674(04)00128-x.

Sato, T.K., Panda, S., Miraglia, L.J., Reyes, T.M., Rudic, R.D., McNamara, P., Naik, K.A., Fitzgerald, G.A., Kay, S.A., Hogenesch, J.B., 2004. A functional genomics strategy reveals Rora as a component of the mammalian circadian clock. Neuron 43, 527—537, http://dx.doi.org/10.1016/j.neuron.2004.07.018.

Scheer, F.A.J.L., Hilton, M.F., Mantzoros, C.S., Shea, S.A., 2009. Adverse metabolic and cardiovascular consequences of circadian misalignment. Proc. Natl. Acad. Sci. U.S.A. 106, 4453—4458, http://dx.doi.org/10.1073/pnas.0808180106.

Scott, A., Monk, T., Brink, L., 1997. Shiftwork as a risk factor for depression: a pilot study. Int. J. Occup. Environ. Health 3 (Suppl. 2), S2—S9.

Selby, C.P., Sancar, A., 2006. A cryptochrome/photolyase class of enzymes with single-stranded DNA-specific photolyase activity. Proc. Natl. Acad. Sci. U.S.A. 103, 17696—17700, http://dx.doi.org/10.1073/pnas.0607993103.

Spiegel, K., Tasali, E., Leproult, R., Van Cauter, E., 2009. Effects of poor and short sleep on glucose metabolism and obesity risk. Nat. Rev. Endocrinol. 5, 253—261, http://dx.doi.org/10.1038/nrendo.2009.23.

Stanewsky, R., Kaneko, M., Emery, P., Beretta, B., Wager-Smith, K., Kay, S.A., Rosbash, M., Hall, J.C., 1998. The cryb mutation identifies cryptochrome as a circadian photoreceptor in *Drosophila*. Cell 95, 681—692, http://dx.doi.org/10.1016/s0092-8674(00)81638-4.

Stone, J.R., Yang, S., 2006. Hydrogen peroxide: a signalling messenger. Antioxid. Redox Signal. 8, 243—270, http://dx.doi.org/10.1089/ars.2006.8.243.

Sugano, S., Andronis, C., Green, R.M., Wang, Z.Y., Tobin, E.M., 1998. Protein kinase CK2 interacts with and phosphorylates the *Arabidopsis* circadian clock-associated 1 protein. Proc. Natl. Acad. Sci. U.S.A. 95, 11020—11025, http://dx.doi.org/10.1073/pnas.95.18.11020.

Tamai, T.K., Carr, A.J., Whitmore, D., 2005. Zebrafish circadian clocks: cells that see light. Biochem. Soc. Trans. 33, 962—966, http://dx.doi.org/10.1042/bst20050962.

Tamaru, T., Hattori, M., Ninomiya, Y., Kawamura, G., Varès, G., Honda, K., Mishra, D.P., Wang, B., Benjamin, I., Sassone-Corsi, P., Ozawa, T., Takamatsu, K., 2013. ROS stress resets circadian clocks to coordinate pro-survival signals. PLOS ONE 8, 1—16, http://dx.doi.org/10.1371/journal.pone.0082006.

Vaidya, A.T., Top, D., Manahan, C.C., Tokuda, J.M., Zhang, S., Pollack, L., Young, M.W., Crane, B.R., 2013. Flavin reduction activates *Drosophila* cryptochrome. Proc. Natl. Acad. Sci. U.S.A. 110 (51), 20455—20460, http://dx.doi.org/10.1073/pnas.1313336110.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M., Telser, J., 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol. 39, 44—84, http://dx.doi.org/10.1016/j.biocel.2006.07.001.

van Ooijen, G., Millar, A.J., 2012. Non-transcriptional oscillators in circadian timekeeping. Trends Biochem. Sci. 37, 484—492, http://dx.doi.org/10.1016/j.tibs.2012.07.006.

Wagner, E., Deitzer, G.F., Fischer, S., Frosh, S., Kempf, O., Stroebele, L., 1975. Endogenous oscillations in pathways of energy transduction as related to circadian rhythmicity and photoperiodic control. Biosystems 7, 68—76, http://dx.doi.org/10.1016/0303-2647(75)90044-1.

Wang, T.A., Yu, Y.V., Govindaiah, G., Ye, X., Artinian, L., Coleman, T.P., Sweedler, J.V., Cox, C.L., Gillette, M.U., 2012. Circadian rhythm of redox state regulates excitability in suprachiasmatic nucleus neurons. Science 337, 839—842, http://dx.doi.org/10.1126/science.1222826.

Wood, T.L., Bridwell-Rabb, J., Kim, Y.-I., Gao, T., Chang, Y.-G., Li Wang, A., Barondeau, S.S., 2010. The KaiA protein of the cyanobacterial circadian oscillator is modulated by a redox-active cofactor. Proc. Natl. Acad. Sci. U.S.A. 107, 5804—5809, http://dx.doi.org/10.1073/pnas.0910141107.

Yang, Y., Cheng, P., Liu, Y., 2002. Regulation of the *Neurospora* circadian clock by casein kinase II. Genes Dev. 16, 994—1006, http://dx.doi.org/10.1101/gad.965102.

Yang, Y., He, Q., Cheng, P., Wrage, P., Yarden, O., Liu, Y., 2004. Distinct roles for PP1 and PP2A in the *Neurospora* circadian clock. Genes Dev. 18, 255—260, http://dx.doi.org/10.1101/gad.1152604.

Yoshida, Y., Iigusa, H., Wang, N., Hasunuma, K., 2011. Cross-talk between the cellular redox state and the circadian system in *Neurospora*. PLoS ONE 6, e28227, http://dx.doi.org/10.1371/journal.pone.0028227.

Yoshii, K., Ishijima, S., Sagami, I., 2013. Effects of NAD(P)H and its derivatives on the DNA-binding activity of NPAS2, a mammalian circadian transcription factor. Biochem. Biophys. Res. Commun. 437, 386—391, http://dx.doi.org/10.1016/j.bbrc.2013.06.086.

Yoshii, K., Tajima, F., Ishijima, S., Sagami, I., 2015. Changes in pH and NADPH regulate the DNA binding activity of neuronal PAS domain protein 2, a mammalian circadian transcription factor. Biochemistry 54, 250—259, http://dx.doi.org/10.1021/bi5008518.

Zhang, R., Lahens, N.F., Ballance, H.I., Hughes, M.E., Hogenesch, J.B., 2014. A circadian gene expression atlas in mammals: implications for biology and medicine. Proc. Natl. Acad. Sci. U.S.A. 111, 16219—16224, http://dx.doi.org/10.1073/pnas.1408886111.

Zoltowski, B.D., Vaidya, A.T., Top, D., Widom, J., Young, M.W., Crane, B.R., 2011. Structure of full-length *Drosophila* cryptochrome. Nature 480, 396—399, http://dx.doi.org/10.1038/nature10618.